EXHIBIT B15
Part 1

Personal Information

Black n' Red

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9/26/2017

- Cell lines

SKOV-3	ATCC
A2780	Sigma Aldrich, St. Louis MO
TOV112	A kind gift from Genshong Wu at Wayne State Ur

EL-1 / macrophages

Normal Ovarian epithelial Cell Biologic, Chicago, IL

- Fetal bovine serum (FBS, Innovative Research, Novi, MI)
Penicilin/streptomycin (Fisher Scientific)

- Talc (Fisher # T4-500 Lot #166820)

78

Seeded Cells for PCR

9/26/2017

— Thawing Cells

	Media
EL-1 (Macrophages)	IMDM (10% FBS, 1% PS, 1ml H-T, 2μL
Normal Ovarian Epithelial	Complete Human Epithelial Cell medium k (Cell Biologics)
SKOV-3	McCoy's 5A (10% FBS, 1% PS)
TOV112	Medium 199: MCDB 105 (1:1) + 10% FBS +
AL780	RPMI-1640 (10% FBS + 1% PS)

75 cm² flask + 15 mL medium

9/29/2017

— Subculture cells

※ Check ~~the~~ under microscop cells are 70~80% full.

① Sork out old medium
② Wash with 10mL PBS
③ gently remove PBS
④ pipet trypsin-EDTA 2mL onto the washed Cells mondarger
※ Normal Ovarian Epithelial use trypsn from ScienCell
⑤ 37°C incubator 1~5 minutes (SKOV-3 longer)
⑥ Check under microscope
⑦ Add fresh medium 8mL to inactive trypsin. Then mix
⑧ Take 2mL to a new 100mm dish
⑨ Add 8mL Fresh medium to 100mm dish
⑩ Incubate the cells

※ One time ~~treat~~ ~~one cell~~ One Cell line.

10/3/2017

- Subculture Cells

  2mL Cells + 8mL medium 100 mm dish

  Cells doubled in one day

10/6/2017

- Subculture Cells

- Seeded Cells for talc treat

  1×10^6 cells/dish 60mm dish + 5mL medium

10/7/2017

- Treat cells with talc

  Prepare talc

  100mg talc + 10ml DMSO → mix 10 mg/mL = 10^4 µg/mL

- sterilization under UV light to avoid endotoxin and microbial Contamination

$(x_1)(10^4\ \mu g/mL) = (5mL)(20\ \mu g/mL) \rightarrow x_1 = 10\ \mu L$

$(x_2)(10^4\ \mu g/mL) = (5mL)(100\ \mu g/mL) \rightarrow x_2 = 50\ \mu L$

$(x_3)(10^4\ \mu g/mL) = (5mL)(1000\ \mu g/mL) \rightarrow x_3 = 500\ \mu L$

ini

10/10/2017
After 72 hours treatment

— Collect cells

Cell Collection Protocal

Put on gloves and spray with 70% ethanol
Remove cell culture dish from incubator
Observe cells under microscope.
Move the dishes to your work bench, does not need to be done in the hood.
Collect media and place in labeled 15ml tube for freezing,
Add 10 ml PBS
Using a cell scraper, scrape the bottom of the dish and rotate it to ensure scraping of entire bottom
Using a 10ml pipet, remove the PBS and cell mixture and place into the 15ml conical centrifugation tube that corresponds to the dish, 1ml for RNA, 2ml for DNA,8 ml for protein assay.
Close and centrifuge all tubes, 5 minutes at 1800rpm (slower speed keeps cells from breaking).
Place another paper towel by sink, dump PBS from all tubes into sink and place tubes upside down to drain them. Cells will be collected at the bottom. Place all tubes in Styrofoam holder and place in -80°C freezer.

— RNA Extraction
RNeasy Mini kit (Qiagen Cat # 74106) (go to pg 42. 43)

— Detect Concentration of RNA by Nanodrop
(Thermo Fisher Scientific)
(go pg 43)

— cDNA Synthesis via Reverse Transcription — VILO kit
Life Technolog
(go pg 43)

RNA Extraction

RNeasy Mini Kit (Qiagen cat # 74106)

Important Notes before starting: WORK IN THE HOOD

- β-Mercaptoethanol (β-ME) can be added to Buffer RLT (lysis buffer) before use. β-ME is toxic; dispense in a fume hood and wear appropriate protective clothing. Add 10 µl β-ME per 1 ml Buffer RLT. Buffer RLT is stable for **one month** after addition of β-ME.
- Buffer RPE is supplied as a concentrate. Before using for the first time, add ethanol as indicated on the bottle. Be sure to **mark the lid with a X** to show that the working solution has been prepared.

Buffer RW1 and Buffer RLT are hazardous.

- **Buffer RLT+ β-ME should be disposed of in the jar in the hood.**
- **Buffer RWI should be disposed of in the jar in the hood.**

Preparation of the Buffer RLT

- In a labeled 15ml centrifugation tube, add 10µl β-ME for every 1 ml Buffer RLT.

Preparation of your samples

1. Add 350 µl of the Buffer RLT + β-ME solution to each of your sample tubes.
    a. if you have a lot of cells, you will need to add 600 µl of Buffer RLT + β-ME solution to each tube ***also add equal volume of ethanol)
2. Add 350 µl of 70% ethanol to each tube and pipet to mix
3. Transfer the entire sample to its corresponding mini spin column
    a. Close columns and place them into the small centrifuge.
    b. Centrifuge the tubes for 15 seconds at 13,000 rpm
4. Dump the flow through into hazardous waste jar **in the hood**.
5. Add 700µl of the Buffer RW1 to the RNeasy column
    a. Centrifuge 15 seconds at 13,000 rpm
6. Dump the flow through into hazardous waste jar **in the hood**
7. Add 500µl of Buffer RPE onto each RNeasy column
    a. Centrifuge 15 seconds at 13,000 rpm
8. Dump the flow through into waste jar
9. Add 500µl Buffer RPE to each column again
    a. Centrifuge 2 minutes at 13,000 rpm to dry the silica gel membrane
10. Dump the flow through in waste jar, centrifuge for one minute more
11. Remove columns from collection tubes and place in corresponding 1.5ml centrifuge tube
12. Add 50µl of RNase-free water to each column, onto the center of the silica-gel membrane without touching the sides of the column (water dissolves RNA).
    a. Allow to stand for 1 minute
    b. Centrifuge columns for 1 minute at 13,000 rpm, LID MUST BE ON CENTRIFUGE
13. Collect flow through from the collection tube and place back into the column on the center of the membrane, allow to stand for 1 minute
    a. Centrifuge columns again for 1 minute at 13,000 rpm, LID MUST BE ON CENTRIFUGE
14. Remove and dispose of columns
15. Place your microcentrifuge tubes containing RNA on ice
    a. Detect concentration of RNA
    b. Good quality RNA has a A260/A280 of 2.0

NEED TO MEASURE RNA EACH TIME YOU GO TO MAKE cDNA

cDNA Synthesis via Reverse Transcription

You will need:
Ice
Thaw, on ice:
RNA
VILO MasterMix
RNase-free water

You must detect the concentration of your RNA. After doing this, you can calculate the volume needed to get for a 1 µg reaction.
i.e. – If your RNA concentration is 0.9 ug/ul then:
(x ul)(0.9 ug/ul) = 1 ug solve for x

For a single reaction, combine the following components in a sterile PCR tube on ice.

	1 µg RNA
Component	**Volume/reaction**
VILO MasterMix	4 µl
Template RNA	Variable up to 1 µg
RNase-free Water	Variable
Total Volume:	20 µl

The total amount in each tube should equal **20 ul**, hence the variable volume of water.

- Add 4 ul VILO MasterMix to each tube, volume of RNA calculated, volume of water calculated, and gently mix.
- Place the tubes in a rack and the rack into a 25°C water bath for 10 minutes.
- Place the rack into a 42°C water bath for 60 minutes.
- Then, place racked tubes into 85°C water bath for 5 minutes to terminate the reaction.
- Place samples on ice for a few minutes.
- Centrifuge cDNA.
- Place into -80°C freezer for storage or continue on.

Do 0.2ug Reaction

Sample	Concentration ug/ul RNA	ul RNA for 0.2 ug in 1.5 ug rxn	µl Water
SKOV unt 72 hr	0.0521	3.8	20.2
SKOV talc 20ug/ml 72 hr	0.0431	4.6	19.4
A2780 unt 72 hr	0.0976	2.0	22.0
A2780 talc 20 ug/ml 72 hr	0.1067	1.9	22.1
EL1 72 hr	0.0067	24.0	0.0
EL1 talc 20ug/ml 72 hr	0.0146	11.0	13.0
SKOV talc 100ug/ml 72 hr	0.086	2.3	21.7
SKOV talc 1000ug/ml 72 hr	0.0592	3.4	20.6
A2780 talc100ug/ml 72 hr	0.0289	6.9	17.1
A2780 talc 1000ug/ml72 hr	0.0335	6.0	18.0
EL1 talc 100ug/ml 72 hr	0.0104	15.5	8.5
EL1 talc1000ug/ml 72 hr	0.0128	12.6	11.4
Normal OV Epi 72 hr	0.0433	4.6	19.4
Normal OV Epi talc 20ug/ml72 hr	0.0385	5.2	18.8
Normal Ov Epi talc 100ug/ml72 hr	0.0357	5.6	18.4
Normal Ov Epi talc 1000ug/ml72 hr	0.0667	3.0	21.0

0.2µg RNA was obtained from each sample following dilution as described by this table

DNA (30ng) prepared

1/2

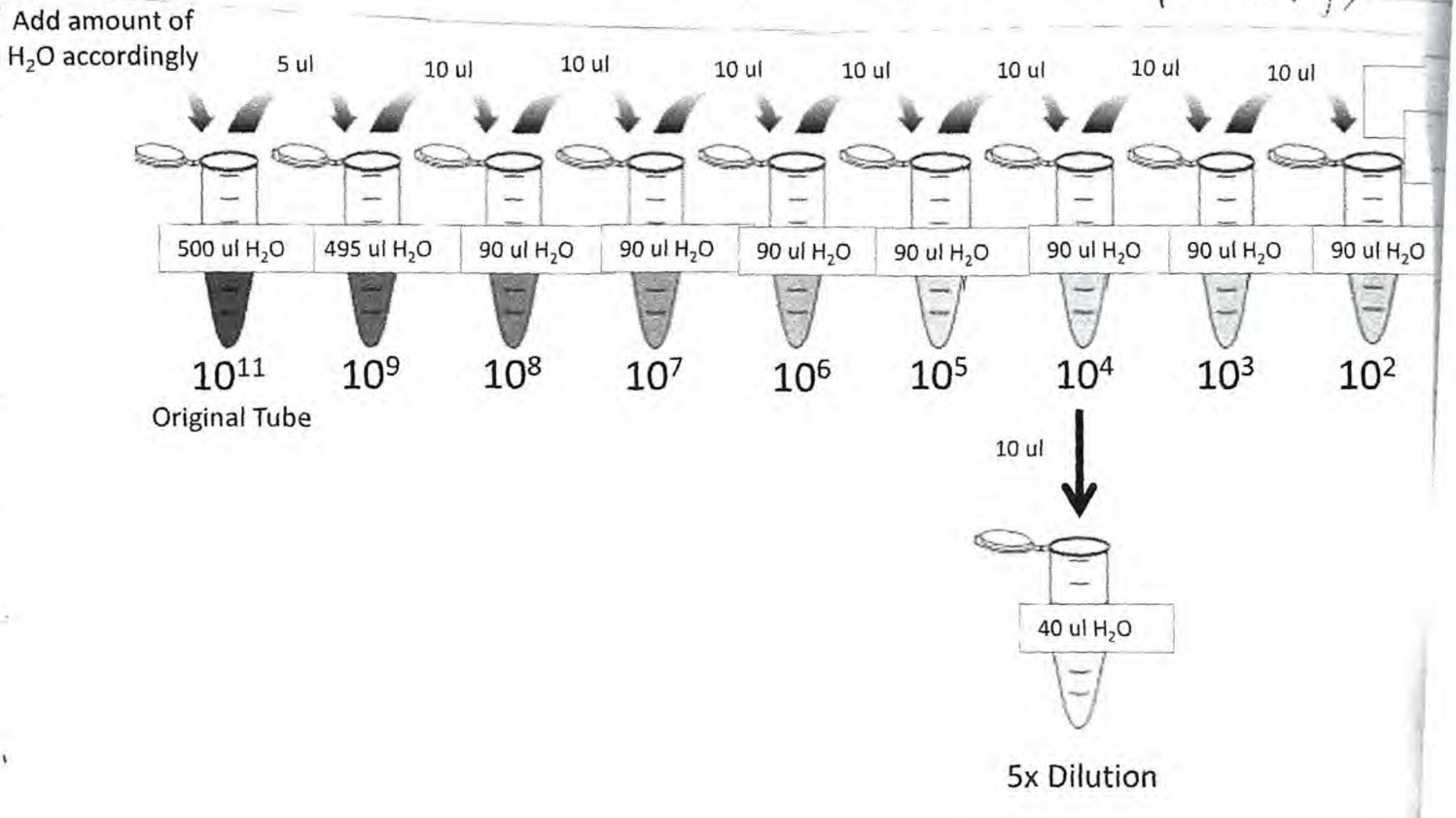
10/11/2017 Real-time PCR for β-actin

β-actin – Standard

– Standards come desiccated

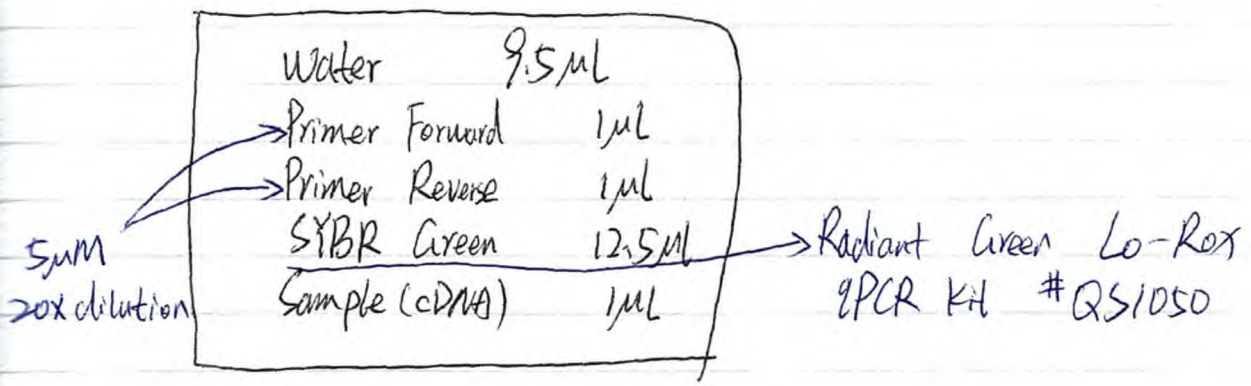
- Reconstitute the standard using TE buffer
- The volume of TE buffer is on the product sheet
  ① You will add TE buffer such that the concentration will be 100μM
  - Mix well
  ② In a new 1.5mL microtube, add 5μl of standard to each tube
  - Calculate the number of tubes needed by dividing the volume of TE buffer you added by 5
  ③ put tubes into the concentrator machine for 2minutes – Lids open
  ④ Close tubes, label the lid with the type of standard and date
  - The box should state that user add 500μl of PCR water to get a standard that is 10^{11}

– Serial Dilution of Standard (place samples on ice after mixing)



Run β-actin with samples

— Do 25µl reaction



— Calculating Master mix for samples

20 samples X 3 (triplicated) + 1 blank = 61
61 X 1.17 extra = 71.37

— Master mix calculation

Water = 9.5 X 71.37 = 678.015µL = 678µL
primer = 1 X 71.37 = 71.4µL
SYBR Green = 12.5 X 71.37 = 892.125 = 892.1

— Mix, then take out 80.6µL of this mix ⟶ 1.5mL tube/~~per~~ sample
24 X 3 X 1.12 extra = 80.6µL

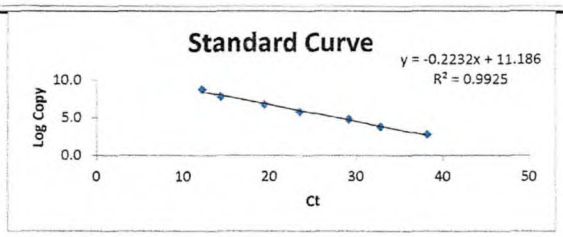
— Add 3.4µl Sample to 1.5mL tube containing master mix
1 X 3 X 1.12 = 1.4µL

— Mix well add 25µL ⟶ PCR tube
3 total per sample

β-actin Gene information

Gene of Interest	B-actin		
		Unit	Formula
1 Dalton = 1.66E-24	1.66E-24	g	
Mass of base pair	615	Da	
Avg. Mass/base	305.25	Da	
Length of entire	79	bases	
Mass in Daltons	2.41E+04	Da	= number bases x avg. mass/base
Mass in grams	4.00E-20	g	= mass in Da x mass of a Da in grams
Mass in ug	4.00E-14	ug	= above / 10E-6
Mass in ng	4.00E-11	ng/copy	= above x 10E3

Copy #	Ct	Log Copy #
610000000	12.29	8.8
61000000	13.15	7.8
6099999.5	16.12	6.8
610000	20.69	5.8
61000	24.74	4.8
6100	28.15	3.8
610	31.71	2.8



Oligonucleotide primers and cycling condition

Accession Number	Gene	Sense (5'-3')	Antisense (3'-5')	Amplicon (bp)	Annealing Time (sec) and Temperature (°C)
NM_001101	β-actin	ATGACTTAGTTGCGTTACAC	AATAAAGCCATGCCAATCTC	79	10, 64
NM_001752	CAT	GGTTGAACAGATAGCCTTC	CGGTGAGTGTCAGGATAG	105	10, 63
NM_000637	GSR	TCACCAAGTCCCATATAGAAATC	TGTGGCGATCAGGATGTG	116	10, 63
NM_000581	GPX1	GGACTACACCCAGATGAAC	TTCTCCTGATGCCCAAAC	96	10, 61
NM_000852	GSTp1	TACCAGTCCAATACCATC	GTAGATGAGGGAGATGTA	138	10, 57
NM_000250	MPO	CACTTGTATCCTCTGGTTCTTCAT	TCTATATGCTTCTCACGCCTAGTA	79	60, 63
NM_000625	NOS2	GAGGACCACATCTACCAGGAGGAG	CCAGGCAGGCGGGAATAGG	89	30, 59
NM_003102	SOD3	GTGTTCCTGCCTGCTCCT	TCCGCCGAGTCAGAGTTG	84	60, 64

An initial cycle was performed at 95°C followed by 35 cycles of denaturation at 95°C for 15 seconds, annealing temperature and time per the table, followed by extension cycle at 72°C for 30 seconds.

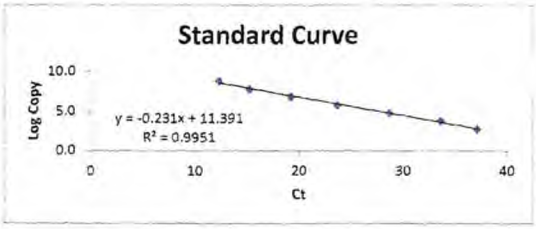
10/13/2017

Run Real-time PCR - GSR with standard & samples

Gene of Interest	GSR		
		Unit	Formula
1 Dalton = 1.66E-24	1.66E-24	g	
Mass of base pair	615	Da	
Avg. Mass/base	305.25	Da	
Length of entire	103	bases	
Mass in Daltons	3.14E+04	Da	= number bases x avg. mass/base
Mass in grams	5.22E-20	g	= mass in Da x mass of a Da in grams
Mass in ug	5.22E-14	ug	= above / 10E-6
Mass in ng	5.22E-11	ng/copy	= above x 10E3

Gene information

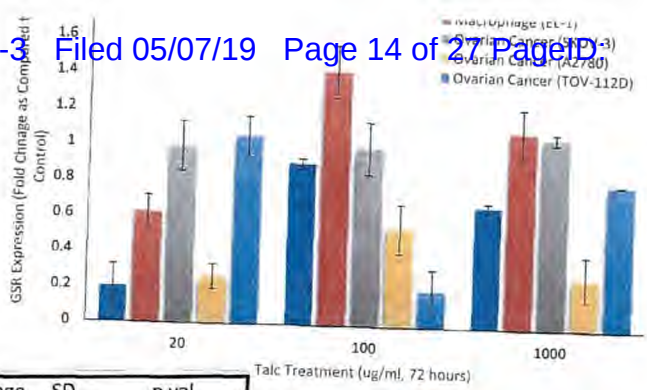
Copy #	Ct	Log Copy #
607000000	12.29	8.8
60700000	13.15	7.8
6070000	16.12	6.8
607000	20.69	5.8
60700	24.74	4.8
6070	28.15	3.8
607	31.71	2.8



Standard Curve

Data

Normal Ov Epithelial Cells	fg/ul cDNA	Fold Change	Average	SD	p val
Normal Ov Epithelial -Control	4.46428128	4.252409179			
	4.040537078				
	5.649374711				
Normal Ov Epithelial 20 ug/ml Talc	3.436513604	-0.191866667	0.200031	0.122962	0.3818
	4.733285555	0.11308328			
	5.472758654	0.286978375			
Normal Ov Epithelial 100 ug/ml Talc	8.010433347	0.883740019	0.904084	0.028771	0.0493
	8.183455957	0.924428156			
	6.552998884	0.541008545			
Normal Ov Epithelial 1000 ug/ml Talc	7.045842037	0.656905942	0.674378	0.02471	0.0633
	7.629372716	0.794129491			
	7.194442969	0.691851058			
EL-1 Cells	fg/ul cDNA	Fold Change	Average	SD	p val
EL1 Control DMSO (5 ug/ml volume)	57.54265341	56.14675379			
	56.08810465				
	54.80950331				
EL1 20 ug/ml Talc	94.65256367	0.685806521	0.619541	0.093714	0.05
	103.7557756	0.847938992			
	87.21138337	0.553275612			
EL1 100 ug/ml Talc	130.0248721	1.315803912	1.418008	0.144539	0.05
	117.4033866	1.09100934			
	141.5017433	1.520212368			
EL1 1000 ug/ml Talc	125	1.226308585	1.095698	0.133677	0.0042
	110	0.959151555			
	118	1.101635305			



SKOV-3 Cells	fg/ul cDNA	Fold Change	Average	SD	p val
SKOV control for 20 ug/ml Talc	114.1745767	120.5460773			
	127.061285				
	120.4023703				
SKOV-3 Control for 100 ug/ml Talc	101.1965313	103.1239404			
	105.0513496				
	74.08540446				
SKOV-3 20 ug/ml	193.1882215	0.602608942	0.988734	0.138282	0.0825
	227.9470905	0.890954028			
	251.5211034	1.08651421			
SKOV-3 100 ug/ml	228.5877349	1.216631114	0.993998	0.146443	0.043
	216.3075686	1.09754949			
	194.9503956	0.890447502			
SKOV-3 control for 1000 ug/ml Talc	8.767536762	8.745966216			
	12.50147198				
	8.72439567				
SKOV-3 1000 ug/ml Talc	18.23385621	1.084830396	1.063699	0.029884	0.0112
	11.80474342	0.349735768			
	17.86422909	1.042567813			

A2780 Cells	fg/ul cDNA	Fold Change	Average	SD	p val
A2780 control for 20 ug/ml Talc	140.1662906	131.4102463			
	133.6702915				
	129.1502011				
A2780 Control for 100 ug/ml Talc	59.80237268	58.6912448			
	59.17958401				
	57.09177772				
A2780 20 ug/ml	217.2079848	0.652899914	0.26082	0.068898	0.0766
	172.0867487	0.309538286			
	159.2825972	0.212101808			
A2780 100 ug/ml	96.49799078	0.644163301	0.546833	0.137646	0.1088
	85.07310653	0.449502508			
	114.628098	0.953069805			
A2780 control for 1000 ug/ml Talc	3.268388429	5.483649208			
	7.698909987				
	0.370810318				
A2780 1000 ug/ml Talc	5.048597924	-0.079336089	0.282275	0.124705	0.5365
	6.547999324	0.194095223			
	7.515090464	0.370454269			

TOV112 Cells	fg/ul cDNA	Fold Change	Average	SD	p val
TOV112 Control for 20 ug/ml Talc	72.41291598	72.18860045			
	71.52391916				
	72.62896621				
TOV112 20 ug/ml Talc	155.3169283	1.151543697	1.051945	0.108838	0.0035
	139.7405248	0.935769968			
	149.3235813	1.068520242			
TOV112 control for 100 talc	5.996678626	7.837259573			
	7.53579081				
	9.979309283				
TOV112 100 ug/ml Talc	10.08078007	0.286263391	0.203338	0.117274	0.4114
	8.78096925	0.120413222			
	7.925913775	0.011311888			
TOV112 Control for 1000 tov	7.753431026	7.785682592			
	7.825356753				
	7.778259997				
TOV112D 1000 Talc 48 hr	14.05672982	0.805458886	0.806772	0.001857	0.0026
	14.07717885	0.808085378			

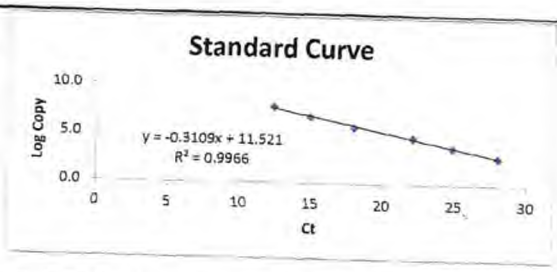
10/16/2017

Run Real-time PCR – iNOS with Standard & Samples

Gene information

Gene of Interest	iNOS		
		Unit	Formula
1 Dalton = 1.66E-24	1.66E-24	g	
Mass of base pair	615	Da	
Avg. Mass/base	305.25	Da	
Length of entire	89	bases	
Mass in Daltons	2.72E+04	Da	= number bases x avg. mass/base
Mass in grams	4.51E-20	g	= mass in Da x mass of a Da in grams
Mass in ug	4.51E-14	ug	= above / 10E-6
Mass in ng	4.51E-11	ng/copy	= above x 10E3

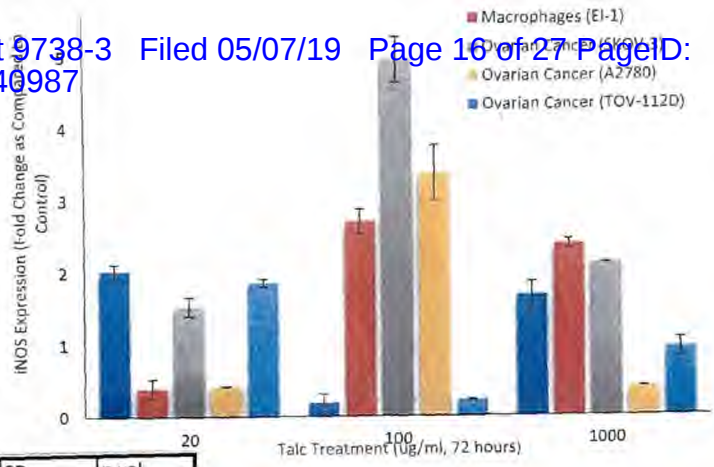
Copy #	Ct	Log Copy #
61500000	12.29	7.8
6150000	13.15	6.8
615000	16.12	5.8
61500	20.69	4.8
6150	24.74	3.8
615	28.15	2.8



Standard Curve

Normal Ov Epithelial Cells	fg/ul cDNA	Fold Change	Average	SD	p val
Normal Ov Epithelial -Control for 1000	0.024815	0.023762852			
	0.241742				
	0.022711				
Normal Ov Epithelial -Control for 20 100	0.126806	0.183761711			
	0.093339				
	0.240718				
Normal Ov Epithelial 20 ug/ml Talc	0.119802	4.041569265	2.018802	0.095183	0.0351
	0.070136	1.951497602			
	0.073335	2.086107216			
Normal Ov Epithelial 100 ug/ml Talc	0.234882	0.278189205	0.191452	0.122665	ns
	0.152123	-0.172171608			
	0.203004	0.10471476			
Normal Ov Epithelial 1000 ug/ml Talc	0.0606	1.550198328	1.677861	0.180542	0.065
	0.076926	2.237232983			
	0.066667	1.805523796			
EL-1 Cells	fg/ul cDNA	Fold Change	Average	SD	p val
EL1 Control DMSO for 20 ug/ml	0.031913	0.032005997			
	0.033565				
	0.032099				
EL1 Control DMSO for 100 ug/ml	0.092988	0.109375796			
	0.131096				
	0.125764				
EL1 20 ug/ml Talc	0.041749	0.304399045	0.395871	0.129361	0.11
	0.047604	0.487343726			
	0.164327	4.134252728			
EL1 100 ug/ml Talc	0.392141	2.585260966	2.702807	0.166235	0.0135
	0.417854	2.820352453			
	0.029852	-0.72706732			
EL-1 1000 control	0.867264	0.946591901			
	0.881998				
	1.02592				
EL-1 1000 ug/ml Talc	3.243944	2.426971659	2.382584	0.062774	0.0135
	3.159909	2.338196032			

Data



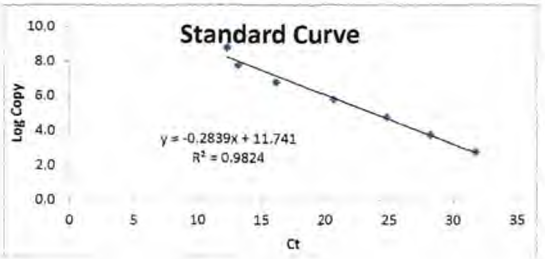
SKOV-3 Cells	fg/ul cDNA	Fold Change	Average	SD	p val
SKOV control for 20 ug/ml Talc	0.018948	0.01936476			
	0.015705				
	0.019781				
SKOV-3 Control for 100 ug/ml Talc	0.013424	0.011345283			
	0.015051				
	0.009267				
SKOV-3 20 ug/ml	0.047144	1.434516565	1.52669	0.130353	0.0294
	0.050714	1.618863155			
	0.144431	6.458419298			
SKOV-3 100 ug/ml	0.06	4.288541523	4.949609	0.31163	0.05
	0.065	4.729253316			
	0.07	5.16996511			
SKOV-3 control for 1000 ug/ml Talc	1.369745	1.01247397			
	1.137957				
	0.655203				
SKOV-3 1000 ug/ml Talc	2.310336	1.281871867	2.117303	0.003559	0.0396
	3.15364	2.114786159			
	3.158736	2.119819883			
A2780 Cells	fg/ul cDNA	Fold Change	Average	SD	p val
A2780 control for 20 ug/ml Talc	0.094243	0.079147127			
	0.068008				
	0.064051				
A2780 Control for 100 ug/ml Talc	0.053171	0.051076582			
	0.048683				
	0.048982				
A2780 20 ug/ml	0.112398	0.420119005	0.424255	0.005849	0.0629
	0.118215	0.493609261			
	0.113053	0.428390528			
A2780 100 ug/ml	0.209538	3.102432407	3.368828	0.37674	0.05
	0.180167	2.527397018			
	0.236751	3.635223784			
A2780 control for 1000 ug/ml Talc	4.549583	4.548883598			
	3.933995				
	4.548184				
A2780 1000 ug/ml Talc	6.369001	0.400123916	0.40196	0.002597	0.0032
	6.385709	0.403796873			
	6.86353	0.508838338			
TOV112 Cells	fg/ul cDNA	Fold Change	Average	SD	p val
TOV112 Control for 20 ug/ml Talc	0.058522	0.052902734			
	0.058744				
	0.047283				
TOV112 Control 1000 volume	0.062537	0.064595234			
	0.068004				
	0.063245				
TOV112 20 ug/ml Talc	0.159626	2.017356105	1.854482	0.054431	0.0133
	0.148974	1.815992924			
	0.153046	1.892970354			
TOV112 100 ug/ml Talc	0.064349	0.216369215	0.222484	0.008648	0.0224
	0.064996	0.228599616			
	0.046148	-0.127677239			
TOV112 Control for 1000	0.053966	0.05318876			
	0.04459				
	0.06101				
TOV112D 1000 ug/ml Talc	0.078515	0.476157328	0.947326	0.121924	0.1077
	0.09899	0.861112589			
	0.108161	1.03353966			

10/16/2017 Run RT-PCR – GPX with standard & samples

Gene of Interest	GPX		
		Unit	Formula
1 Dalton = 1.66E-24	1.66E-24	g	
Mass of base pair	615	Da	
Avg. Mass/base	305.25	Da	
Length of entire	96	bases	
Mass in Daltons	2.93E+04	Da	= number bases x avg. mass/base
Mass in grams	4.86E-20	g	= mass in Da x mass of a Da in grams
Mass in ug	4.86E-14	ug	= above / 10E-6
Mass in ng	4.86E-11	ng/copy	= above x 10E3

Gene information

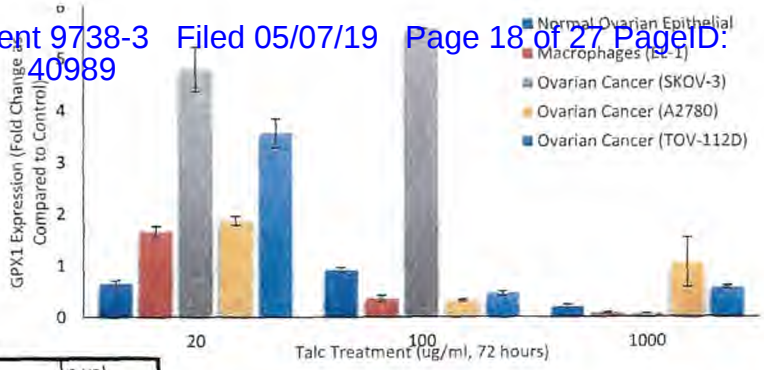
Copy #	Ct	Log Copy #
609000000	12.29	8.8
60900000	13.15	7.8
6090000	16.12	6.8
609000	20.69	5.8
60900	24.74	4.8
6090	28.15	3.8
609	31.71	2.8



Standard Curve

Data

Normal Ov Epithelial Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
Normal Ov Epithelial -Control for 1000	10.44452	12.39509			
	12.94154				
	13.79921				
Normal Ov Epithelial -Control for 20 100	12.52692	12.89282			
	13.25873				
	18.81577				
Normal Ov Epithelial 20 ug/ml Talc	25.05152	0.943059	0.640542	0.059069	0.0694
	21.68973	0.682311			
	20.61271	0.598774			
Normal Ov Epithelial 100 ug/ml Talc	24.93692	0.93417	0.902286	0.045092	0.0425
	24.11475	0.870401			
	21.4644	0.664833			
Normal Ov Epithelial 1000 ug/ml Talc	16.43495	0.325924	0.194533	0.042818	0.325
	15.18163	0.22481			
	14.43106	0.164256			
EL-1 Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
EL1 Control DMSO for 20 ug/ml	26.43411	25.98751			
	24.94745				
	26.58098				
EL1 Control DMSO for 100/1000 ug/ml	26.2594	26.26166			
	26.03356				
	26.26393				
EL1 20 ug/ml Talc	66.79229	1.570168	1.644264	0.104787	0.0394
	70.6434	1.718359			
	77.07548	1.965866			
EL1 100 ug/ml Talc	36.51237	0.39033	0.345133	0.063918	0.0742
	46.80624	0.782303			
	34.13847	0.299935			
EL1 1000 regular	28.5	0.085232	0.075712	0.013463	0.0414
	28	0.066193			
	27	0.028115			



SKOV-3 Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
SKOV control for 20 ug/ml Talc	6.817514	7.415123			
	7.716939				
	7.710917				
SKOV-3 Control for 100 ug/ml Talc	8.439988	8.412195			
	8.384401				
	8.053921				
SKOV-3 20 ug/ml	32.91981	3.439549	4.783855	0.421067	0.0315
	40.68022	4.486115			
	45.09577	5.081594			
SKOV-3 100 ug/ml	55.1	5.550015	5.555959	0.008406	0.0207
	52.1	5.19339			
	55.2	5.561902			
SKOV-3 control for 1000 ug/ml Talc	54.53351	52.15021			
	49.76691				
	39.85461				
SKOV-3 1000 ug/ml Talc	35.33473	-0.32244	0.070767	0.000524	ns
	55.82142	0.070397			
	55.86005	0.071138			
A2780 Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
A2780 control for 20 ug/ml Talc	19.61021	20.77316			
	21.9361				
	16.44827				
A2780 Control for 100 ug/ml Talc	8.250935	8.317047			
	8.383159				
	6.165789				
A2780 20 ug/ml	23.21169	1.790857	1.854858	0.090511	0.0192
	24.27628	1.918858			
	21.72254	1.611809			
A2780 100 ug/ml	10.74368	0.291767	0.307519	0.022277	0.0162
	11.00571	0.323272			
	12.73299	0.530951			
A2780 control for 1000 ug/ml Talc	0.33411	0.215358			
	0.223734				
	0.206982				
A2780 1000 ug/ml Talc	0.368504	0.711124	1.047611	0.475865	0.291
	0.513434	1.384098			
	0.0282	-0.86906			
TOV112 Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
TOV112 Control for 20 ug/ml Talc	15.60499	15.52105			
	16.04456				
	15.43712				
TOV112 Control 100 talc	5.87752	15.09463			
	14.00668				
	16.18258				
TOV112 20 ug/ml Talc	73.71172	3.749145	3.552248	0.278454	0.01
	67.59964	3.355352			
	59.39598	2.826801			
TOV112 100 ug/ml Talc	22.41454	0.484934	0.453197	0.044883	0.0465
	21.45642	0.42146			
	19.28461	0.27758			
TOV112 Control for 1000ug/ml Talc	4.893757	7.868342			
	7.055626				
	8.681058				
TOV112D 1000 Talc	14.70037	0.868293	0.56558	0.030965	0.1387
	12.49081	0.587476			

10/17/2017 Run RT-PCR – SOD3 with standard & samples

Gene information

Gene of Interest	SOD3	
		Unit
1 Dalton = 1.66E-24	1.66E-24	g
Mass of base pair	615	Da
Avg. Mass/base	305.25	Da
Length of entire	85	bases
Mass in Daltons	2.59E+04	Da = number bases x avg. mass/base
Mass in grams	4.31E-20	g = mass in Da x mass of a Da in grams
Mass in ug	4.31E-14	ug = above / 10E-6
Mass in ng	4.31E-11	ng/copy = above x 10E3

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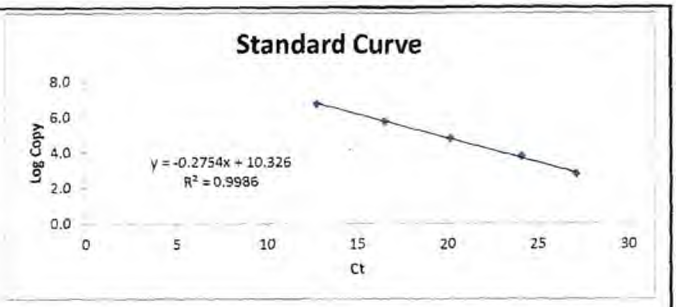
67000000	13.15	7.8
6099999.5	16.12	6.8
610000	20.69	5.8
61000	24.74	4.8
6100	28.15	3.8
610	31.71	2.8

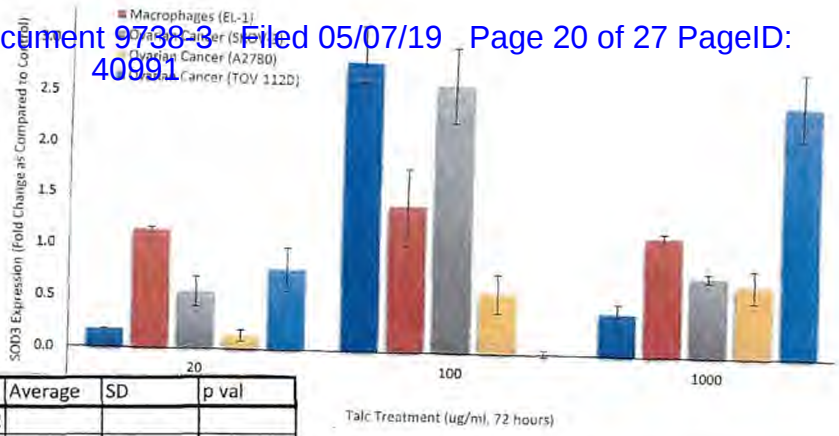


Standard Curve

Data

Normal Ov Epithelial Cells	fg/ul cDNA	Fold Change	Average	SD	p val
Normal Ov Epithelial -Control for 1000	0.069583	0.060875247			
	0.102165				
	0.052168				
Normal Ov Epithelial -Control for 200 500	1.025561	0.635120216			
	0.679916				
	0.590325				
Normal Ov Epithelial 20 ug/ml Talc	0.749187	0.17959921	0.179165	0.000614	0.2374
	0.748636	0.178730864			
	0.803527	0.265158094			
Normal Ov Epithelial 100 ug/ml Talc	3.517144	4.537760335	2.831552	0.347194	0.05
	2.277572	2.586048719			
	2.589421	3.077055842			
Normal Ov Epithelial 1000 ug/ml Talc	0.082194	0.350206214	0.4135	0.089512	0.05
	0.116134	0.907744026			
	0.0899	0.476794623			

EL-1 Cells	fg/ul cDNA	Fold Change	Average	SD	p val
EL1 Control DMSO for 20 ug/ml	0.018405	0.01823182			
	0.017237				
	0.019054				
EL1 Control DMSO for 100/1000 ug/ml	0.110426	0.118911669			
	0.085674				
	0.127398				
EL1 20 ug/ml Talc	0.038627	1.118643876	1.137817	0.027115	0.0021
	0.0348	0.908736299			
	0.039326	1.156990307			
EL1 100 ug/ml Talc	0.254701	1.141934765	1.411269	0.380896	0.0887
	0.318755	1.680603474			
	0.380953	2.203659803			
EL1 1000 ug/ml Talc	0.26	1.186496936	1.147252	0.042327	0.0629
	0.25	1.1024009			
	0.256	1.152858521			



SKOV-3 Cells	fg/ul cDNA	Fold Change	Average	SD	p val
SKOV control for 20 ug/ml Talc	0.006913	0.010297182			
	0.013535				
	0.010443				
SKOV-3 Control for 100 ug/ml Talc	0.012234	0.011557716			
	0.010882				
	0.050575				
SKOV-3 20 ug/ml	0.019103	0.652865731	0.551015	0.144039	0.05
	0.016749	0.449164129			
	0.022063	0.908963284			
SKOV-3 100 ug/ml	0.038926	2.367972796	2.626045	0.364969	0.05
	0.044892	2.884116792			
	0.026396	1.283859584			
SKOV-3 control for 1000 ug/ml Talc	0.208612	0.1401244			
	0.151128				
	0.129121				
SKOV-3 1000 ug/ml Talc	0.244017	0.741431658	0.770192	0.040674	0.04
	0.23448	0.673367285			
	0.252077	0.798953236			

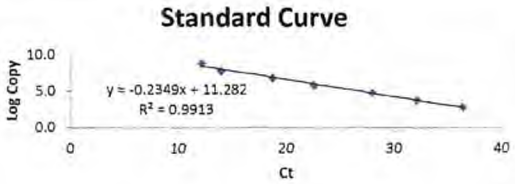
A2780 Cells	fg/ul cDNA	Fold Change	Average	SD	p val
A2780 control for 20 ug/ml Talc	0.029232	0.021280566			
	0.028951				
	0.021281				
A2780 Control for 100 ug/ml Talc	0.039562	0.027201344			
	0.026435				
	0.027968				
A2780 20 ug/ml	0.028102	0.320560377	0.133381	0.055191	0.187
	0.024949	0.172406621			
	0.023288	0.09435509			
A2780 100 ug/ml	0.046663	0.715466188	0.584546	0.18515	0.1692
	0.039541	0.453625014			
	0.058702	1.15805571			
A2780 control for 1000 ug/ml Talc	0.052637	0.099050365			
	0.098587				
	0.099513				
A2780 1000 ug/ml Talc	0.178792	0.805058554	0.696194	0.153958	0.1029
	0.157225	0.587328489			
	0.068449	-0.308951611			

TOV112 Cells	fg/ul cDNA	Fold Change	Average	SD	p val
TOV112 Control for 20 ug/ml Talc	0.030712	0.030110321			
	0.043831				
	0.029509				
TOV112 Control 100 talc	0.016775	0.014654626			
	0.016117				
	0.011072				
TOV112 20 ug/ml Talc	0.03435	1.343995658	0.77735	0.209132	0.1204
	0.028214	0.925229068			
	0.023879	0.629471258			
TOV112 100 ug/ml Talc	0.014484	-0.011613672	0.011113	0.032141	ns
	0.015151	0.033840165			
	0.018271	0.246757232			
TOV112 Control for 1000ug/ml Talc	0.031325	0.028505848			
	0.025687				
	0.068399				
TOV112D 1000 Talc	0.106165	2.724327168	2.490101	0.331245	0.05
	0.47479	15.65588928			

10/18/2017 Run RT-PCR CAT with standard & samples

Gene of Interest	CAT		
		Unit	Formula
1 Dalton = 1.66E-24	1.66E-24	g	
Mass of base pair	615	Da	
Avg. Mass/base	305.25	Da	
Length of entire	105	bases	
Mass in Daltons	3.21E+04	Da	= number bases x avg. mass/base
Mass in grams	5.32E-20	g	= mass in Da x mass of a Da in grams
Mass in ug	5.32E-14	ug	= above / 10E-6
Mass in ng	5.32E-11	ng/copy	= above x 10E3

Copy #	Ct	Log Copy #
606000000	12.29	8.8
60600000	13.15	7.8
6060000.5	16.12	6.8
606000	20.69	5.8
60600	24.74	4.8
6060	28.15	3.8
606	31.71	2.8



Normal Ov Epithelial Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
Normal Ov Epithelial -Control for 1000	0.255112	0.277963			
	0.300814				
Normal Ov Epithelial -Control for 200 500	0.275147	0.196178			
	0.264911				
	0.196178				
Normal Ov Epithelial 20 ug/ml Talc	0.23504	0.198092	0.266425	0.096638	0.161
	0.162371	-0.17233			
	0.261851	0.334759			
Normal Ov Epithelial 100 ug/ml Talc	0.629433	2.208474	2.006022	0.28631	0.05
	0.139599	-0.28841			
	0.55	1.80357			
Normal Ov Epithelial 1000 ug/ml Talc	0.263472	-0.05213	0.197083	0.057972	0.1312
	0.32135	0.15609			
	0.344139	0.238076			
EL-1 Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
EL1 Control DMSO for 20 ug/ml	29.20198	28.39182			
	22.84908				
	27.58165				
EL1 Control DMSO for 100/1000 ug/ml	31.24367	30.90921			
	30.57474				
	33.16323				
EL1 20 ug/ml Talc	25.06856	-0.11705	0.51189	0.047031	0.0765
	41.98112	0.478634			
	43.86952	0.545146			
EL1 100 ug/ml Talc	20.73672	-0.32911	-0.33013	0.001441	0.0189
	17.25388	-0.44179			
	20.67373	-0.33115			
EL1 1000 ug/ml Talc	21	-0.32059	-0.31897	0.002288	0.0189
	21.1	-0.31736			
	22	-0.28824			

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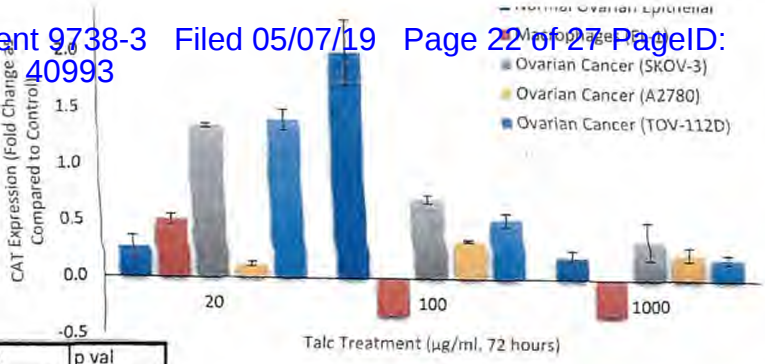

SKOV-3 Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
SKOV control for 20 ug/ml Talc	2.474985	3.537794			
	3.399213				
	3.676375				
SKOV-3 Control for 100 ug/ml Talc	5.576323	5.393164			
	5.152521				
	5.450649				
SKOV-3 20 ug/ml	9.01118	1.547119	1.34984	0.017963	0.0245
	8.358183	1.362541			
	8.268313	1.337138			
SKOV-3 100 ug/ml	8.554721	0.586215	0.705385	0.035231	0.0462
	9.331777	0.730297			
	9.063065	0.680473			
SKOV-3 control for 1000 ug/ml Talc	14.71117	14.67545			
	14.63973				
	13.97333				
SKOV-3 1000 ug/ml Talc	15.39131	0.048779	0.347002	0.167419	0.2053
	21.50518	0.465385			
	18.03053	0.228619			

A2780 Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
A2780 control for 20 ug/ml Talc	6.448358	5.673473			
	5.853449				
	5.493497				
A2780 Control for 100 ug/ml Talc	4.163294	3.876415			
	3.989297				
	3.763532				
A2780 20 ug/ml	7.589043	0.337636	0.121263	0.021465	0.2349
	6.275344	0.106085			
	6.447571	0.136442			
A2780 100 ug/ml	5.154843	0.329797	0.337465	0.010844	0.0691
	5.979143	0.542442			
	5.214293	0.345133			
A2780 control for 1000 ug/ml Talc	9.973024	9.842133			
	11.24123				
	8.312149				
A2780 1000 ug/ml Talc	11.69434	0.188192	0.230282	0.059524	ns
	12.52286	0.272372			
	16.00005	0.625669			

TOV112 Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
TOV112 Control for 20 ug/ml Talc	3.382153	3.406415			
	3.150577				
	3.686515				
TOV112 Control 100 talc	0.838564	3.664997			
	3.735731				
	3.594263				
TOV112 20 ug/ml Talc	8.42336	1.472793	1.408291	0.091219	0.0134
	7.983921	1.34379			
	9.243696	1.713614			
TOV112 100 ug/ml Talc	2.319637	0.291634	0.528072	0.058693	0.0419
	2.818786	0.569574			
	2.66972	0.48657			
TOV112 Control for 1000ug/ml Talc	1.807987	1.795893			
	1.783799				
	2.391376				
TOV112D 1000 Talc	2.186972	0.217763	0.189448	0.040043	0.0722
	2.085271	0.161133			
	1.779704	-0.00901			

10/18/2017 Run RT-PCR MPO with standard & samples

Gene of Interest	MPO		
		Unit	Formula
1 Dalton = 1.66E-24	1.66E-24	g	
Mass of base pair	615	Da	
Avg. Mass/base	305.25	Da	
Length of entire	79	bases	
Mass in Daltons	2.41E+04	Da	= number bases x avg. mass/base
Mass in grams	4.00E-20	g	= mass in Da x mass of a Da in grams
Mass in ug	4.00E-14	ug	= above / 10E-6
Mass in ng	4.00E-11	ng/copy	= above x 10E3

Gene information

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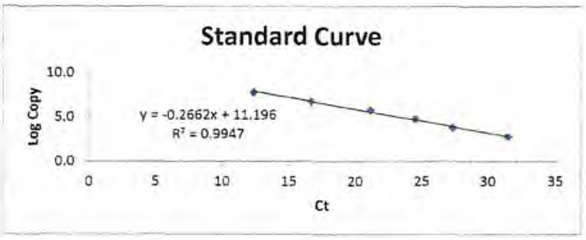
60900000	12.29	7.8
6090000	13.15	6.8
609000	16.12	5.8
60900	20.69	4.8
6090	24.74	3.8
609	28.15	2.8



Normal Ov Epithelial Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
Normal Ov Epithelial -Control for 1000	0.003502	0.003044			
	0.00298				
	0.003108				
Normal Ov Epithelial -Control for 200 500	0.003502	0.003044			
	0.00298				
	0.003108				
Normal Ov Epithelial 20 ug/ml Talc	0.006317	1.075409	1.206998	0.186096	0.05
	0.007118	1.338587			
	0.009902	2.253146			
Normal Ov Epithelial 100 ug/ml Talc	0.006142	1.017918	0.962795	0.077956	ns
	0.007321	1.405213			
	0.005807	0.907672			
Normal Ov Epithelial 1000 ug/ml Talc	0.006317	1.075409	1.206998	0.186096	0.05
	0.007118	1.338587			
	0.009902	2.253146			
EL-1 Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
EL1 Control DMSO (5 ug/ml volume)	0.026276	0.025624			
	0.024419				
	0.026177				
EL1 20 ug/ml Talc	0.035331	0.37884	0.257395	0.044953	0.0242
	0.033034	0.289182			
	0.031405	0.225609			
EL1 100 ug/ml Talc	0.05	0.951307	1.244003	0.137978	0.0101
	0.055	1.146437			
	0.06	1.341568			
EL-1 1000 control	0.00479	0.004725			
	0.004184				
	0.005202				
EL-1 1000 ug/ml Talc	0.011248	1.380253	1.032749	0.491445	0.1629
	0.003024	-0.36008			
	0.007964	0.685245			

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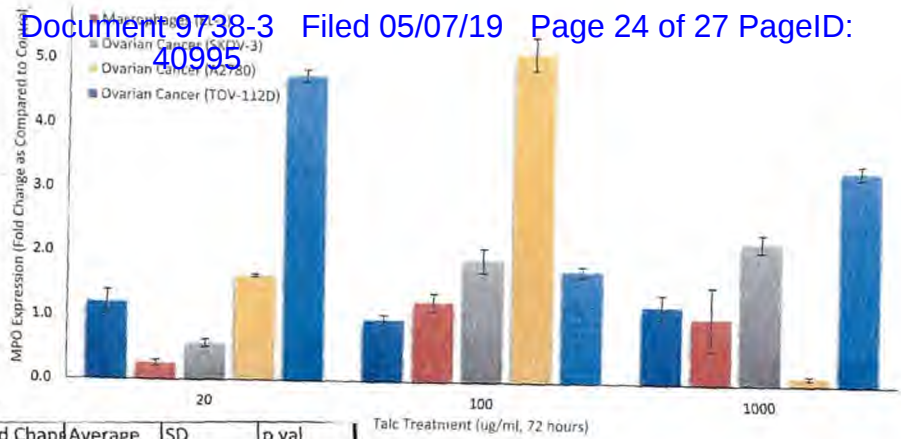


SKOV-3 Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
SKOV control for 20 ug/ml Talc	0.022406	0.021915			
	0.021424				
	0.029877				
SKOV-3 Control for 100 ug/ml Talc	0.025145	0.016548			
	0.014775				
	0.018321				
SKOV-3 20 ug/ml	0.027	0.631639	0.571208	0.060431	0.1817
	0.025	0.510777			
	0.026	0.571208			
SKOV-3 100 ug/ml	0.045764	1.765596	1.897412	0.186416	0.0083
	0.050127	2.029228			
	0.028656	0.731711			
SKOV-3 control for 1000 ug/ml Talc	0.001542	0.001052			
	0.001059				
	0.001044				
SKOV-3 1000 ug/ml Talc	0.001403	0.333859	2.211632	0.136334	0.008
	0.003276	2.115229			
	0.003479	2.308034			

A2780 Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
A2780 control for 20 ug/ml Talc	0.0108	0.01085			
	0.02				
	0.0109				
A2780 Control for 100 ug/ml Talc	0.063463	0.075407			
	0.072816				
	0.089943				
A2780 20 ug/ml	0.028626	1.638359	1.624942	0.018975	0.05
	0.026093	1.40486			
	0.028335	1.611525			
A2780 100 ug/ml	0.064579	4.951951	5.130818	0.252956	0.05
	0.056682	4.224148			
	0.06846	5.309685			
A2780 control for 1000 ug/ml Talc	0.004955	0.004432			
	0.004108				
	0.004233				
A2780 1000 ug/ml Talc	0.004912	0.108294	0.129488	0.029972	0.5279
	0.005751	0.297523			
	0.0051	0.150681			

TOV112 Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
TOV112 Control for 20 ug/ml Talc	0.088068	0.075135			
	0.070801				
	0.066536				
TOV112 Control 100 talc	0.10722	0.108482			
	0.097003				
	0.121222				
TOV112 20 ug/ml Talc	0.042682	5.583981	4.748687	0.092276	0.05
	0.036844	4.683438			
	0.03769	4.813937			
TOV112 100 ug/ml Talc	0.019449	2.000104	1.742775	0.075626	0.05
	0.017434	1.689299			
	0.018127	1.79625			
TOV112 Control for 1000ug/ml Talc	0.008193	0.006483			
	0.005154				
	0.0061				
TOV112D 1000 Talc	0.014068	1.170051	3.334727	0.107619	0.05
	0.027607	3.258629			

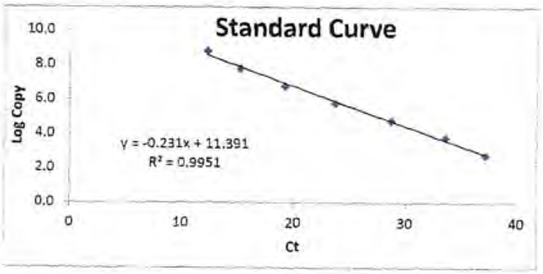
10/19/2017 Run RT-PCR GSTp1 with Standard & samples

Gene of Interest	GSTp1		
		Unit	Formula
1 Dalton = 1.66E-24	1.66E-24	g	
Mass of base pair	615	Da	
Avg. Mass/base	305.25	Da	
Length of entire	100	bases	
Mass in Daltons	3.05E+04	Da	= number bases x avg. mass/base
Mass in grams	5.07E-20	g	= mass in Da x mass of a Da in grams
Mass in ug	5.07E-14	ug	= above / 10E-6
Mass in ng	5.07E-11	ng/copy	= above x 10E3

Gene information

Standard Curve

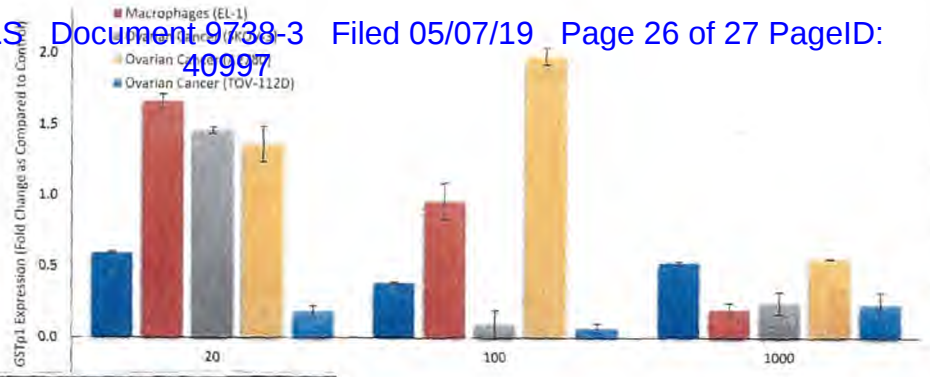
Copy #	Ct	Log Copy #
606000000	12.29	8.8
60600000	13.15	7.8
6060000.5	16.12	6.8
606000	20.69	5.8
60600	24.74	4.8
6060	28.15	3.8
606	31.71	2.8



Data

Normal Ov Epithelial Cells	fg/ul cDNA	Fold Chan	Average	SD	p val
Normal Ov Epithelial -Control for 1000	4.5	4.42			
	4.4				
	4.44				
Normal Ov Epithelial -Control for 200 500	4.5	4.42			
	4.4				
	4.44				
Normal Ov Epithelial 20 ug/ml Talc	7	0.58371	0.592006	0.007273	0.003
	7.05	0.595023			
	7.06	0.597285			
Normal Ov Epithelial 100 ug/ml Talc	6.1	0.38009	0.385747	0.007999	0.004
	6.05	0.368778			
	6.15	0.391403			
Normal Ov Epithelial 1000 ug/ml Talc	6.8	0.538462	0.527149	0.011312	0.05
	6.7	0.515837			
	6.75	0.527149			

EL-1 Cells	fg/ul cDNA	Fold Chan	Average	SD	p val
EL1 Control DMSO (5 ug/ml volume)	10.20286	10.36977			
	9.75591				
	11.15053				
EL1 Control DMSO (1000 ug/ml volume)	34.79645	33.68753			
	32.57861				
	68.16306				
EL1 20 ug/ml Talc	24.71735	1.383597	1.665897	0.049681	0.0051
	28.00903	1.701027			
	27.28045	1.630768			
EL1 100 ug/ml Talc	19.38792	0.869658	0.959908	0.127633	0.0711
	21.25967	1.050158			
	33.05055	2.187203			
EL1 1000 ug/ml Talc	41.59777	0.234812	0.201666	0.046876	0.0007
	39.36456	0.16852			
	33.1334	-0.01645			



SKOV-3 Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
SKOV control for 20 ug/ml Talc	36.31595	35.95677			
	35.5976				
	68.59786				
SKOV-3 Control for 100 ug/ml Talc	72.75467	61.72977			
	58.02745				
	65.4321				
SKOV-3 20 ug/ml	87.92087	1.445182	1.461944	0.023705	0.0116
	89.12626	1.478706			
	84.90128	1.361204			
SKOV-3 100 ug/ml	71.91819	0.165049	0.09506	0.098979	ns
	63.27742	0.025071			
	32.76049	-0.46929			
SKOV-3 control for 1000 ug/ml Talc	4.842595	4.837675			
	6.244395				
	4.832755				
SKOV-3 1000 ug/ml Talc	5.783046	0.195418	0.249358	0.076282	0.1381
	3.650555	-0.24539			
	6.304928	0.303297			

A2780 Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
A2780 control for 20 ug/ml Talc	59.49055	30.29235			
	34.42553				
	26.15917				
A2780 Control for 100 ug/ml Talc	12.54541	13.77486			
	13.56743				
	13.9823				
A2780 20 ug/ml	31.33308	1.274657	1.362953	0.12487	0.0342
	33.76562	1.451249			
	25.47339	0.849267			
A2780 100 ug/ml	9.139274	1.941376	1.983008	0.058877	0.05
	9.397987	2.02464			
	7.876923	1.535102			
A2780 control for 1000 ug/ml Talc	2.663943	3.107142			
	3.176421				
	3.481062				
A2780 1000 ug/ml Talc	4.842595	0.558537	0.556953	0.002239	0.0661
	6.244395	1.009691			
	4.832755	0.55537			

TOV112 Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
TOV112 Control for 20 ug/ml Talc	20.39414	20.7106			
	16.15828				
	21.02706				
TOV112 Control 100 talc	20.17126	19.40386			
	22.03957				
	18.63647				
TOV112 20 ug/ml Talc	27.94732	0.349421	0.186779	0.037984	0.0394
	24.02265	0.15992			
	25.13518	0.213638			
TOV112 100 ug/ml Talc	21.15492	0.090243	0.068337	0.030979	ns
	16.15982	-0.16719			
	20.30481	0.046431			
TOV112 Control for 1000ug/ml Talc	5.996679	6.766235			
	7.535791				
	9.979309				
TOV112D 1000 Talc	10.08078	0.489866	0.234578	0.089358	ns
	8.780969	0.297763			

WAYNE STATE
School of Medicine

Talcum Powder Enhances Oxidative Stress in Ovarian Cancer Cells

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BACKGROUND

- We have previously characterized epithelial ovarian cancer (EOC) cells to manifest a persistent pro-oxidant state as evident by the upregulation of certain key oxidant and downregulation of key antioxidant enzymes.
  - This redox state is further enhanced in chemoresistant EOC cells.
- Several studies have suggested a possible association between genital use of talcum powder and risk of EOC; however, the biologic basis for this association has yet to be delineated.

OBJECTIVE

To determine the effects of talcum powder on the expression of key oxidant and antioxidant enzymes in EOC cells.

METHODS

- **Cell Culture:** Human ovarian cancer cell lines, SKOV-3 (HTB-77) and TOV-112D (CRL-11731), as well as human macrophages (EL-1, CRL-9854) were all obtained from American Type Culture Collection (ATCC). The ovarian cancer cell line A2780 was obtained from Sigma Aldrich. Human primary ovarian surface epithelium cells from Cell Biologics. Cells were seeded in 60mm² culture dishes (1.0×10^6) and allowed to rest for 24 hours.
- **Cell Treatment:** Talcum powder was obtained from Sigma Aldrich and was prepared in DMSO. Cell lines were treated with talcum powder (0, 20, 100, 1000 µg/ml) for 72 hours. Additionally, talc was soaked in DMSO for 72 hours, spun down, and supernatant collected and was used to treat cells (1000 µg/ml, referred to as "supernatant").
- **Real-time RT-PCR Analysis:** Total RNA was isolated from cells utilizing a RNeasy Extraction Kit (Qiagen). cDNA synthesis was performed using the SuperScript VILO Master Mix Kit (Life Technologies). Quantitative real-time RT-PCR was performed using a QuantiTect SYBR Green RT-PCR kit (Qiagen) and a Cepheid 1.2f Detection System. A standard with a known concentration was designed specifically for β-actin, MPO, iNOS, CAT, SOD3, GSR, GPX, GSTp1 using the Beacon Designer software. This allowed for absolute quantification of gene expression as copy numbers per microgram of RNA. Following real-time RT-PCR, a melting curve analysis was performed to demonstrate the specificity of the PCR product as a single peak. All samples were normalized to β-actin. A control, which contained all the reaction components except for the template, was included in all experiments.
- **Statistical Analysis:** Data were analyzed using SPSS 23.0 for Windows. Data was analyzed with one way ANOVA followed by Tukey's post hoc tests with Bonferroni correction.

RESULTS

There was a marked increase in mRNA levels of the pro-oxidant enzymes, iNOS and MPO in talc treated ovarian cancer cell line, macrophages, and normal ovarian epithelial cells, all as compared to their control (Figure 1A&B). Additionally, there was a marked increase in the mRNA levels of the antioxidant enzymes CAT, SOD3, GSR, GPX1 and GSTp1, in talc treated ovarian cancer cell lines and in normal ovarian epithelial cells, all compared to their control (Figures 1&2). Interestingly, macrophages had decreased CAT mRNA levels at the 100, 1000, and supernatant doses (Figure 2D).

RESULTS

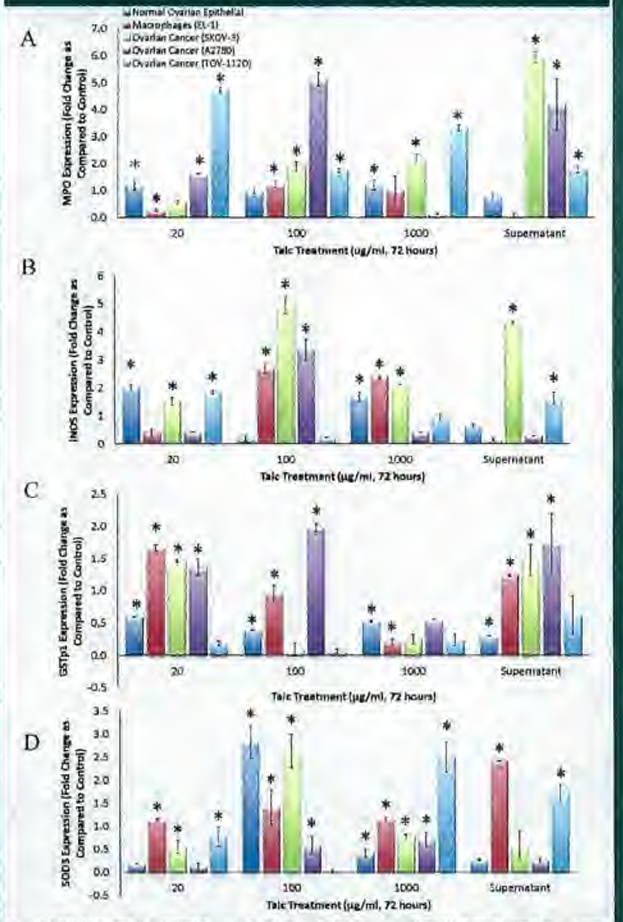

Figure 1: MPO, iNOS, GSTp1, and SOD3 Expression in Talc Treated Cells. Expression of MPO (A), iNOS (B), GSTp1 (C), and SOD3 (D) mRNA levels in normal ovarian epithelial cells, macrophages, and ovarian cancer cell lines (SKOV-3, A2780, and TOV-112D) after treatment with talc (20, 100, 1000, and supernatant from 1000 µg/ml) for 72 hours was determined by real-time RT-PCR. Fold change was calculated as compared to control. *P<.05 vs. controls.

RESULTS

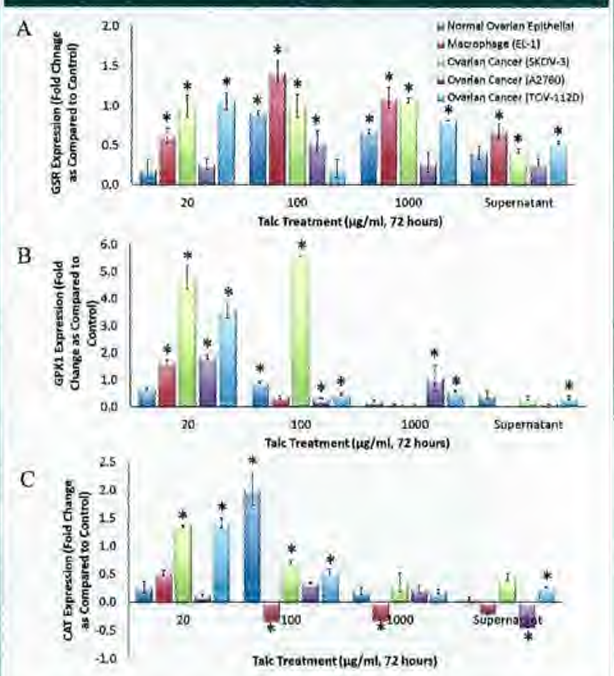

Figure 2: GSR, GPX1, and CAT Expression in Talc Treated Cells. Expression of GSR (A), GPX1 (B), SOD3 (C) and CAT (D) mRNA levels in normal ovarian epithelial cells, macrophages, and ovarian cancer cell lines (SKOV-3, A2780, and TOV-112D) after treatment with talc (20, 100, 1000, and supernatant from 1000 µg/ml) for 72 hours was determined by real-time RT-PCR. Fold change was calculated as compared to control. *P<.05 vs. controls.

CONCLUSIONS

This is the first report to show that talcum powder induces a biological effect by further enhancing the redox state in normal macrophages and ovarian epithelial cells as well as in ovarian cancer cells. The results of this study will provide a molecular basis to previous reports that link genital use of talcum powder to increased risk of epithelial ovarian cancer.